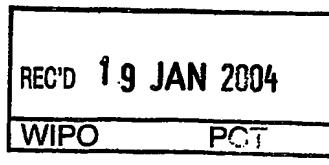




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Dated 16 December 2003

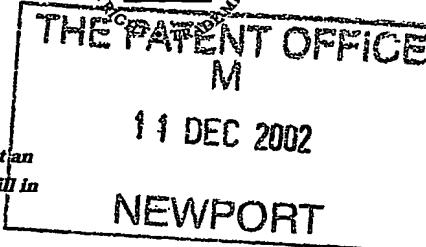
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 Cardiff Road
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1. Your reference PU02105

2. Patent application number
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0228830.6

11 DEC 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

 AMERSHAM BIOSCIENCES AB
 Bjorkgatan 30
 SE 751 84 Uppsala
 Sweden

Patents ADP number (if you know it)

8249120001

If the applicant is a corporate body, give the country/state of its incorporation

Sweden

ES

4. Title of the invention

METHOD AND DEVICE FOR MASS SPECTROSCOPY

5. Name of your agent (if you have one)

 ROLLINS, Anthony, John; HAMMER, Catriona, MacLeod and
 HAMMETT, Audrey, Grace, Campbell
 Amersham plc
 Amersham Place
 Little Chalfont
 Buckinghamshire HP7 9NA

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Country

Priority application number
(if you know it)Date of filing
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

Yes

- a) any applicant named in part 3 is not an inventor, or
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See note (d))

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Description	5	/
Claim(s)	2	/
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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

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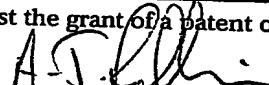
Request for substantive examination
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Any other documents
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11.

I/We request the grant of a patent on the basis of this application.

Signature



Date

10 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom FRANKS, Barry
01494 542196

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Field of the Invention

The present invention relates to devices and methods of the type mentioned in the preambles of the independent claims for performing matrix-assisted laser desorption/ionisation
5 (MALDI) mass spectrometry.

Prior Art

Matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry is a method in which
10 a crystallised matrix made of light-absorbing small molecules is excited by a short laser pulse
that creates vibrational movement of the matrix molecules. This movement releases some of
the matrix molecules at the surface, and embedded analyte molecules are also dragged out
into the surrounding vacuum of the ion source. At some point during this process, a fraction
of the analyte and matrix molecules gets singly ionised, and this fraction of molecules is
15 accelerated out of the ion source for mass-to-charge ratio (M/Z) analysis, often in a time-of-
flight (TOF) system.

Before being examined by MALDI mass spectroscopy the analyte being tested has to be
prepared so that it is in a suitable form for MALDI mass spectroscopy. Typically it is
20 prepared in the following way:
the analyte is added to a solution of laser light absorbing matrix;
droplets of the analyte/matrix mixture are then placed on a (stainless steel) MALDI target
slide; and,
the solvent allowed to evaporate leaving spots of crystals of sample/matrix on the target slide.
25

In order to increase the limit of detection it is eligible to minimise the amount of matrix and
gather all sample/matrix material to a small spot. This is can be achieved by having a
hydrophobic target slide surface. However, a problem that occurs with such a method is that
the droplets are not held onto a particular part of the target slide and can move around as the
30 solvent evaporates) leaving the matrix residue at an undefined position. The result is that
during mass spectroscopy large areas of the target slide give very little, or no, signal.
Consequently, the mass spectrometer (or the operator) needs to search for spots that give good
signals, which is often done by using a camera-equipped mass spectrometer to visually locate

crystals, or by just looking at the spectral quality of the mass spectrometer signal while moving the sample around. Neither method is entirely satisfactory as the first method requires the cost of the camera and the use of an operator to look for the sample spot while the second method may also require an operator and is time-consuming as, if the mass spectrometer is 5 not equipped with means for viewing the target slide in situ, the only way of finding a spot is to systematically aim and fire the laser across the whole of the slide until a signal is received indicating that it has hit a spot.

Summary of the Invention

10

According to the present invention, at least some of the problems with the prior art are solved by means of a method having the features present in the characterising part of claim 1 and a device having the features mentioned in the characterising part of claim 4.

15 Brief Description of the Figures

Figure 1 a) shows schematically a plan view of a target slide in accordance with a first embodiment of the present invention;

Figure 1b) shows a section along line A-A in figure 1a); and,

20

Figures 2 a)- 2e) show schematically stages in the evaporation of solvent from a pit on a target slide in accordance with the present invention.

25 Detailed Description of Embodiment Illustrating the Invention

25

A first embodiment of a MALDI target slide 1 in accordance with the present invention is shown in figures 1a) and 1b). Slide 1 comprises is in the shape of a rectangular thin substrate 3, preferably with rounded corners or ends. Substrate 3 may be typically of the order of 7.5 cm long, 8mm wide and 0.5 mm thick and is made of a conducting material such as stainless 30 steel. Substrate 3 has an upper, sample-receiving surface 5 intended to receive drops containing samples of analyte. Sample receiving surface 5 could be the whole of a surface of substrate 3 or may comprises one or more areas on a surface of said substrate, said areas being specially arranged to receive samples, e.g. by being specially treated (for example, by being

coated with a hydrophobic material) or by being arranged in predetermined positions.

Substrate 3 has a bottom surface 7 intended to rest on, or be held in, a sample mount in a MALDI mass spectrometer. The upper surface 3 is provided with at least one, preferably a plurality of, drop positioning pits 9a-9n. These pits are formed in the sample-receiving surface

5 5 by any suitable method, e.g. drilling, stamping, etching, ablation, sand blasting, (laser)cutting, milling, (hot)embossing, punching, casting, moulding, etc. The number n of

pits can be any suitable number, e.g. 1, 2, 4, 8, 16, 32, 64, 96, 384, 1536 etc. These pits 9a-9n are circular and each have a rim 11a-11n with a diameter $\varnothing 1$ (or maximum width $W1$ in the case of non-circular pits) which is preferably substantially smaller (e.g. less than half the

10 diameter of the drop diameter \varnothing_{drop}) than the diameter of a drop \varnothing_{drop} when it is being applied, e.g. pipetted onto, to the slide 1. For example, for a drop with a diameter \varnothing_{drop} of 1

mm, a suitable rim diameter $\varnothing 1$ could be of in the range of 0.5 mm - 0.05 mm, e.g. 0.18 mm. Each pit is preferably at least 5 μm deep and preferably is less than 0.1 mm deep. Pits 9a-9n

15 may have vertical or sloping walls 13a-13n and, in the case of sloping walls the diameter $\varnothing 2$ of the pit bottoms 15a-15n preferably is the same as or smaller than $\varnothing 1$, e.g. 0.16 mm if $\varnothing 1$ is 0.18 mm, in order to make it easier to manufacture. The upper surface 5 (at least in the region surrounding each pit) and pit rims 9a-9n are coated with a (thin) layer 17 of a hydrophobic material such as PTFE, or FEP or the like. Optionally pit walls 13a-13n and bottoms 15a-15n may also be coated by layer 17. Layer 17 can be deposited by any suitable method e.g.

20 laminating, vapour deposition, dipping, spraying, painting, spin coating, or being left as an residue following evaporation of a carrier liquid after having been applied in solution or suspension in the carrier liquid, etc. Layer 17 is preferably thin, cracked or conductive enough to allow the de-coupling of interfering surface charge build-ups so that any negative influence on the functioning of the mass spectrometer caused by them is minimised. Preferably layer 17 is at least as thick as the thickness of a monolayer of the hydrophobic material and is less than 0.5 mm thick. Preferably layer 17 is a continuous layer.

The use of a pit, which has a rim diameter (or width) which is smaller than the diameter of the drop being applied to it, means that the drop is anchored by a surface effect. This is illustrated

30 in figures 2a)-2e) which show schematically stages in the formation of crystals from a drop applied to a slide in accordance with the present invention. Figure 2a) shows a drop 21 on a pit 9. Figure 2b) shows the same drop after some of the liquid in the drop has evaporated and part of the liquid front 22 of the drop has reached the rim 11 of the pit 9. Figure 2c) shows the

same drop after some more of the liquid has evaporated and the liquid front is now the same size as, and in contact with, the rim 11 of the pit 9. At this point it is energetically disadvantageous for the liquid front to move over the rim 11 and descend the wall 13 of the pit 9. Instead, further evaporation just causes the height of the droplet to decrease as 5 shown in figure 2d). This continues until the liquid eventually becomes saturated with the matrix material and crystals 23 are formed as shown in figure 2e).

While the invention has been illustrated by an example in which the pits are circular, any other shapes are also possible e.g. oval, quadratic or irregular shapes.

10

The size of the pits may be adjusted in accordance with the amount of matrix (and analyte) that it is intended to anchor. It is conceivable to provide a target slide in accordance with the present invention with a plurality of pits with different diameters (or maximum widths), e.g. some pits 0.5mm wide, some pits 0.4 mm wide, some 0.37 mm wide, etc. When a drop of 15 matrix and analyte is to be applied to a target slide in accordance with the present invention the user may select an appropriately sized pit to receive the drop. The preferred diameter or width of a pit may be chosen depending on the dry volume of the matrix and analyte in the drop. Preferably the amount of the matrix and analyte in a drop is adjusted, or the size of the pit selected, so that when the solvent evaporates the solid matrix and analyte crystals 20 completely cover the bottom of a pit and more preferably so that the amount of matrix and analyte do not saturate the drop until enough solvent has evaporated for the drop to shrink so that its contact area with the surface is the same as the size of the pit.

25 The optimum size of a size of a pit depends, amongst other, on the amount of solid residue in a drop that it is intended to receive, the size of the overlap area of the laser spot that is used to excite the matrix material and the eye of the sampling ion optics, and also the number of times that the solid residue is intended to be excited by a laser. The optimum size pit for a drop containing a solid volume V of matrix material and analyte would have a maximum diameter (or maximum width) and shape which is exactly the same size as the laser spot/ion optics eye 30 that will be used to excite and sample the matrix/analyte material. Furthermore, it will provide for the distribution of the matrix/analyte material to a surface density such that when the solid residue is analysed, for example by being excited 100 times by the laser, then when the

analysis is finished, most, or all, of the solid residue has been ablated. In this way most or all of the analyte is sampled.

If the amount of matrix and analyte are not known then it could be practical to just use any pit that has a diameter or maximum width which is less than half the original diameter of the drop that it is intended to anchor.

In order to be able to coat the pit with a layer of hydrophobic material, the pit should not be too small nor have a depth to width ratio which makes it difficult to reliably coat the pit walls (and bottom). Preferably a pit is less than 1 mm wide, more preferably less than 0.7 mm wide, even more preferably about 0.1-0.05 mm wide. Preferably a pit is shallow enough so that it does not unduly affect the electrical field that is used to accelerate the ions during operation of the mass spectrometer. Preferably a pit is less than 1 mm deep, more preferably less than 0.5 mm deep and most preferably less than 0.1 mm deep after the hydrophobic coating has been applied to it. As it is the rim of the pit which provides the surface effect that holds a drop in position, the actual depth of the pit does not influence the positioning of a drop once the drop has come into contact with the rim, and therefore the pit can be very shallow – even less than 5 μm deep for very small drops and correspondingly small pits e.g. pits less than about 0.25 mm wide. However, accurately forming such narrow pits may be expensive, therefore, for commercial reasons, it may be preferable to have pits which are deeper than theoretically necessary.

The thickness of the hydrophobic coating depends on the conductivity of the coating material used. It should be adapted so that the resistance through the coating is sufficiently low that it allows normal operation of the mass spectrometer that the slide is used in. As mentioned previously, it may be as thin as it is reliably possible to make e.g. less than 0.5 mm, preferably less than 100 μm and most preferably less than 10 μm . Molecular hydrophobic monolayers (i.e. layers which are the same depth as one molecule of hydrophobic material) are also conceivable – especially for disposable target slides where wear resistance is not an issue. Alternatively, the pits can be machined in a material that is hydrophobic by itself.

30

The above mentioned embodiments are merely intended to illustrate the present invention and are not intended to limit the scope of protection claimed by the following claims.

Claims

1. Method of preparing a target slide for mass spectroscopy analysis comprising the steps of: making at least one pit (9a-9n) having a wall (13a-13n) and a pit bottom (15a-15n) in a sample receiving surface (5) of said target slide, wherein there is a rim (11a-11n) between said sample receiving surface and said wall (13a-13n), wherein said sample receiving surface (5) and the rim (11a-11n) of said at least one pit (9a-9n) are hydrophobic.

5 2. Method in accordance with claim 1 characterised in by the step of making said at least one pit less than 1 mm wide.

10 3. Method in accordance with claim 1 or 2 characterised by the steps of forming said at least one pit (9a-9n) in said slide and coating the rim (11a-11n) of said at least one pit (9a-9n) with a layer of hydrophobic material (17).

15 4. Target slide for use in a mass spectrometer characterised in that it comprises a substrate (3) with a sample receiving surface (5) comprising at least one pit (9a-9n) having a wall (13a-13n) and a pit bottom (15a-15n) in said sample receiving surface (5), wherein there is a rim (11a-11n) between said sample receiving surface and said wall (13a-13n), wherein said sample receiving surface (5) and the rim (11a-11n) of said at least one pit (9a-9n) are hydrophobic.

20 5. Target slide in accordance with claim 4 characterised in that said at least one pit (9a-9n) is less than 1 mm wide.

25 6. Target slide in accordance with claim 4 characterised in that said at least one pit is more than 0.05 mm wide.

7. Target slide in accordance with any of claims 4-6 characterised in that said at least one pit (9a-9n) is less than 100 μm deep.

30 8. Target slide in accordance with any of claims 4-7 characterised in that said at least one pit (9a-9n) is more than 5 μm deep.

9. Target slide in accordance with any of claims 4-8 characterised in that said target slide comprises a substrate (3) of conducting material coated with a layer (17) of hydrophobic material.

5

10. Target slide in accordance with claim 7 characterised in that said layer (17) of hydrophobic material is less than 0.1 mm thick.

Abstract

The present invention relates to mass spectrometer target slides (1) which are provided with pits (9a-9n) on the sample receiving surface (5). The sample receiving surface (5) and the rims (11a-11n) of the pit (9a-9n) are coated with a hydrophobic layer 17. The at least one pit (9a-9n) has a width or diameter $\varnothing 1$ which is less than the width of a drop applied to the pit (9a-9n).

5
Fig. 1

